

Attorney Docket No. 088.000426
U.S. Patent Application No. 09/720,549
Reply to Office Action of September 28, 2004
Date: November 29, 2004

Amendments to the Specification

Please replace paragraph starting at page 13, lines 11-13 as follows:

The amount of induced apoptosis was evaluated by the ratio between the number of apoptotic cells found in the exposure group and the number of apoptotic cells found in the sham-exposed group, that is the group not exposed to the magnetic fields according to the invention.

Please replace paragraph starting at page 13, lines 1-3 as follows:

Human colon adenocarcinoma cell line (WiDr) grown in confluent monolayers in T25 flasks was used for the experiment. For each exposure condition 6 flasks containing each about 10 millions cells were used, 3 exposed and 3 sham-exposed (i.e. not exposed).

Please replace paragraph starting at page 15, lines 8-11 as follows:

As in the example 1 each cell line was grown in confluent monolayers in T25 flasks. The experimental protocol was the same as in example 1. Six flasks (3 exposed and three sham-exposed) for each cell line were exposed for 20 minutes. Apoptosis was evaluated after 3 hours. The exposure conditions used were the R type of Table 1.

Please replace paragraph starting at page 16, lines 8-10 as follows:

In the first experiment, 36 female mice were randomly assigned to 4 experimental groups, each formed by 6 exposed and 3 sham-exposed for a total of 24 animals exposed to 4 different SELF magnetic fields and 12 sham-exposed.

Please replace paragraph starting at page 16, lines 14-17 as follows:

In the second experiment 24 female mice were randomly assigned to 2 experimental groups, formed by 12 exposed to the SELF exposure condition which gave the best results

Attorney Docket No. 088.000426
U.S. Patent Application No. 09/720,549
Reply to Office Action of September 28, 2004
Date: November 29, 2004

among the four exposure conditions used in the previous experiment (exposure condition number 4), and 12 ~~shamesham~~-exposed.

Please replace paragraph starting at page 18, line 1 as follows

The obtained results are reported in Table 3 for the first experiment and in Table 4 for the second.

TABLE 3

exposure conditions	1	2	3	4	shamesha <u>m-</u> exposed
exposure duration (min)	70	70	70	70	-
time averaged field intensity (Static + ELF rms) in mT	3	3	4	6	-
field variation in mT (min-max) Static; [min-max] ELF	(4-6) [2-2]	(1.5-4) [1-1]	(2-5) [1.5-3.5]	(2-5) [1.5-3.5]	-
constant field time duration (min-max) in minutes	(5-15)	(5-20)	(5-15)	(5-20)	-
time % with co-presence of Static and ELF fields	0%	50%	50%	100%	-
S/ELF ratio (min-max)	-	(0,5-5)	(0,5-5)	(0,5-5)	-

Attorney Docket No. 088.000426
 U.S. Patent Application No. 09/720,549
 Reply to Office Action of September 28, 2004
 Date: November 29, 2004

time % with Static field alone	50%	50%	50%	0%	-
number of mice	6	6	6	6	12
extracted tumor mass volume (mm ³)	1323 ± 304	1450 ± 288	920 ± 540	650 ± 205	1492 ± 559
extract tumor mass weight (g)	1.54 ± 0.22	1.6 ± 0.39	0.98 ± 0.56	0.96 ± 0.25	1.6 ± 0.5
number of apoptotic cells per 10 HPF	98 ± 23	115 ± 20	129 ± 25	129 ± 26	40 ± 17
p53 expression per 10 HPF	35.1 ± 0.11	43.8 ± 0.16	38.2 ± 0.06	28.7 ± 0.14	73.2 ± 0.14

TABLE 4

exposure conditions	4 (see tab. 3)	<u>sham</u> <u>sham</u> exposed
number of mice	12	12
extracted tumor mass volume	1139 ± 509 cm ³	1914 ± 793 cm ³
extracted tumor mass weight	1.4 ± 0.7 g	2.1 ± 0.6 g

Attorney Docket No. 088.000426
U.S. Patent Application No. 09/720,549
Reply to Office Action of September 28, 2004
Date: November 29, 2004

apoptosis (assessed in 50% of mice only)	72.5 ± 9.3	37.0 ± 7.4
p53	35.6 ± 6.7	78.1 ± 16.7
proliferative index	0.34 ± 0.08	0.45 ± 0.07
mitosis	24.1 ± 10.9	47.7 ± 10.1

Please replace paragraph starting at page 20, lines 6-8 as follows:

At the histologic examination of 12 organs for each animal for all groups no differences were found between exposed and shamesham-exposed mice. No differences were also found in the blood tests. These findings prove the absence of toxicity related to the SELF fields treatment.

Please replace paragraph starting at page 20, lines 12-16 as follows:

In addition a consistent result is represented by morphological modifications, increase of number and dimensions of mitochondria as well as number of nucleoli, presence of many vacuoles inside the cytoplasm. Non neoplastic cells (i.e. epithelial and stromal cells) showed no differences between exposed and shamesham-exposed animals in agreement with the absence of toxicity found in 12 normal organs examined in each animal.

Please replace paragraph starting at page 21, lines 8-11 as follows:

After the cell inoculation 2 groups of mice were randomly formed respectively of 16 animals exposed and 17 shamesham-exposed. The mice of the former group were exposed 70 minutes once a day, for 5 days a week, for their entire life beginning after 24 hours after the tumor inoculation.

Attorney Docket No. 088.000426
U.S. Patent Application No. 09/720,549
Reply to Office Action of September 28, 2004
Date: November 29, 2004

Please replace paragraph starting at page 21, lines 17-21 as follows:

The antitumor effectiveness of the treatment was evaluated by using the N.C.I. formula: ratio between exposed and shamesham-exposed animals of the average animal life span. This average was evaluated summing for each experimental group the time of survival divided by the number of animals. The effectiveness is obtained when the N.C.I. formula gives as result an index equal or greater than 1.25.

Please replace Table 5 as follows:

TABLE 5

living mice exposed/ <u>shamesham</u> -exp. (days)	16/16 (48)	16/15 (73)	15/14 (76)	14/14 (84)	13/14 (87)	12/14 (88)
living mice exposed/ <u>shamesham</u> -exp. (days)	12/13 (97)	12/12 (107)	10/12 (109)	10/10 (114)	10/9 (115)	9/8 (125)
living mice exposed/ <u>shamesham</u> -exp. (days)	9/7 (149)	8/6 (153)	8/5 (155)	8/4 (157)	7/4 (163)	7/3 (173)
living mice exposed/ <u>shamesham</u> -exp. (days)	6/3 (183)	6/2 (192)	6/0 (194)	5/0 (195)	4/0 (203)	3/0 (257)
living mice exposed/ <u>shamesham</u> -exp. (days)	2/0 (276)	1/0 (323)	0*/0 *sacrificed (326)			

Attorney Docket No. 088.000426
U.S. Patent Application No. 09/720,549
Reply to Office Action of September 28, 2004
Date: November 29, 2004

Please replace paragraph starting at page 22, lines 4-6 as follows:

The N.C.I. formula applied to the results reported in Table 5 gives an index equal to 1.31, that is greater than 1.25 . After 194 days 6 exposed mice were alive whereas all ~~shame~~sham exposed mice were dead.